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FOREWORD

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**Acquired Secondary Events in the
Pathogenesis of Hereditary Breast Cancer**

DAMD17-96-1-6206

Gail E. Tomlinson, M.D.,Ph.D.

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INTRODUCTION: Mutation of the *BRCA1* gene accounts for most families with an inherited predisposition to breast and ovarian cancer, approximately half of families with multiple cases of breast cancer only and approximately 8-10% of women with early-onset breast cancer unselected for family history. (1-3) These observations suggest that inherited *BRCA1* mutations may account for approximately 8-10,000 new cases of breast cancer in the U.S.A. each year. The inheritance of a germline mutation of the *BRCA1* gene, although associated with a markedly increased incidence of breast cancer, is not solely responsible for the development of breast cancer in predisposed women and multiple other acquired steps appear to be required for the development of breast tumors in predisposed women. Some of these observed acquired changes have been described. (4)

Although the function of the *BRCA1* protein is not yet clearly determined, evidence suggests that *BRCA1* may play a role in DNA repair, may function as a transcription factor or may possibly exist as a secreted granin-like molecule. (5-7) If *BRCA1* indeed functions in DNA repair, then one would expect an accelerated accumulation of other genetic aberrations in tumors derived from *BRCA1* mutation carriers. In this project we have identified and begun to characterize *BRCA1* associated tumors derived from *BRCA1* carriers.

No models currently exist for the study of breast cancers associated with *BRCA1* mutations. Somatic mutation of the *BRCA1* is thought not to occur in sporadic breast tumors. (8) Although a modest number of breast cancer cell lines have been established, no breast cancer cell lines have been reported to date which derive from a *BRCA1* predisposed individual. The establishment of such a cell line could not only provide the means of study of the secondary changes which develop during tumor development, tumor growth characteristics conferred by *BRCA1*, and could conceivably serve as a substrate for genetic transfection studies. The work described here has resulted in the characterization of a breast cancer cell line mutant for *BRCA1*.

BODY:**TASK 1**

Patient Selection: Patients have been selected from Registry families for *BRCA1* and *TP53* mutation screening. Families are screened for germline mutation of *BRCA1* if breast and ovarian cancers are observed in first degree relatives or in the same individual or if five or more members of the family have had breast cancer. We have also screened our Registry members who have developed very early onset breast cancer (less than 30 years of age) for both *TP53* and *BRCA1*.

Mutation analysis: SSCP analysis for exons 5 through 9 of the *TP53* gene and exons 2 and 20 of the *BRCA1* gene was performed as a modification of the technique as described by Orita et al (9). Specific genes known to be involved in the pathogenesis of breast cancer were examined as possible secondary acquired changes in the cell line. Coding regions of exons 5 through 11 of the *TP53* gene, the *CDKN2A* gene and the *PTEN* gene and exons 2, 11 and 20 of the *BRCA1* gene were analyzed. (10-14). Primers were designed so as to amplify fragments 150-200 base pairs in length.

Sequence analysis of DNA fragments demonstrating abnormal mobility on SSCP gels was performed by cloning amplified PCR fragments into pCMV5 vectors and sequencing using Sequenase^R according to the manufacturer's instructions. ³⁵S-labeled reactions were electrophoresed on 6% acrylamide gels. A minimum of 8 clones were sequenced for each region of interest.

Direct sequence analysis of the entire coding region of the *BRCA1* gene was done in six individuals thought to be at high-risk of carrying a mutation was done by Myriad Genetics (Salt Lake City, Utah).

During this year we identified only one new family with a *TP53* mutation out of eleven families screened, however we determined mutations of *BRCA1* in seventeen new families. These are in addition to four previously *BRCA1* families previously documented.

Our known *BRCA1* families are listed, along with their mutations below:

Family ID	BRCA1 Mutation
BC73	PTT conf pnd
BC92	3549 C -> T (Term)
BC98	185delAG
BC110	3450del4
BC113	185delAG
BC129	185delAG
BC130	3600del11
BC131	3888delGA
BC132	185insA
BC144	PTT con pnd
BC151	conf pnd
BC201	PTT -conf pnd
BC211	int5spl
BC215	3875del4
BC260	5382insC
BC294	185delAG
BC403	185delAG
BC408	5382insC
BC412	185insA
BC497	185delAG
BC516	185delAG
BC517	185delAG
BC530	185delAG

TASK 2: We have identified tumor blocks from 10 of the mutation carriers listed in the Table above. We have obtained materials from four mutations carriers. (Two tumor specimens from family BC260, one from BC92 and BC215).

TASK 3: The comparison of familial and sporadic breast tumors is a designated to start after month 12.

TASK 4: Using polymorphic dinucleotide and tetranucleotide microsatellite repeat markers, patterns of allelic losses were studied at loci known to be commonly lost in breast cancer. Primer sequences were obtained from the Genome Database. PCR amplification and electrophoresis was performed as described previously. (15)

In one case in which fresh tumor was available, Southern blotting was performed in order to confirm the presence of absence of the *PTEN* coding sequence DNA in tumor cell line as well as constitutional DNA . Genomic DNA was digested overnight with restriction enzymes EcoR1, HindIII, KpnI, BamH1, and MboI. Digested DNA was blotted on Hybond^R membranes according to directions provided by the manufacturer. DNA probes were prepared by amplification of the coding region(s) of the *PTEN* gene. Hybridization with ³²P-labeled probe was carried out using standard techniques. (16)

TASK 5: This task has been eliminated as recommended by the study section reviewers.

CONCLUSION: We have made substantial progress in the identification of subjects who are mutations carriers who will be candidates for characterization of the acquired changes in their tumors. Ongoing recruitment of subjects and identification of their tumor materials is ongoing.

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